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World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1353579

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

August 04, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/590,043*
FILING DATE: *July 20, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/11626*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PROVISIONAL APPLICATION TRANSMITTAL
(37 C.F.R. §1.53(c))

Attorney Docket No. 01656.0010.PZUS00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith for filing under 37C.F.R. §1.53(c) is the provisional patent application of:

Joseph R. Garlich et al.

Title: PTEN INHIBITORS FOR SENSITIZATION OF CANCER CELLS

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"Express Mail" Mailing Label Number

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Date of Deposit July 20, 2004

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Nate Le
(Typed or printed name of person mailing)

Nathan Le
(Signature of person mailing)

PROVISIONAL PATENT APPLICATION TRANSMITTAL

Enclosed are:

1. (X) Cover Sheet for the above-identified provisional patent application identifying the application as a provisional application.
2. **Application Papers Enclosed**

# of Reference pages:		
# of Specification pages:	5	
# of Claims:	1	
# of Abstract pages:		
# of Sheets of Drawings:	29	(X) Formal ( ) Informal

3. **Provisional Application Filing Fee**

( ) $ 160.00 the filing fee for the above-identified provisional patent application ***without*** a claim of small entity status.

(X) $ 80.00 filing fee for the above-identified provisional patent application by an entity claiming small entity status.

4. **Method of Payment of Fees**

( ) Enclosed is our firm check in the amount of: $_____

(X) Charge $ 80.00 Deposit Account No. 08-3038.

5. ( ) A separate written request under 37 C.F.R. §1.136(a)(3) which is a general authorization to treat any concurrent or future reply requiring a petition for an extension of time under 37 C.F.R. §1.136(a) for its timely submission as incorporating a petition for an extension of time for the appropriate length of time therein.

6. (X) The Commissioner is hereby authorized to charge any additional fees which may be required in this application under 37 C.F.R. §§1.16-1.17 during its entire pendency, or credit any overpayment, to Deposit Account No. 08-3038. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 08-3038. This sheet is filed in triplicate.

Please direct all future communications to:

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Attention: IP Prosecution
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2941 Fairview Park Drive
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Respectfully Submitted,

July 20, 2004
(Date)

By:_____
Teddy C. Scott, Jr., Ph.D.
Registration No. 53,573

HOWREY SIMON ARNOLD & WHITE, LLP
321 N. Clark Street, Suite 3400
Chicago, IL 60610
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Fax: (312) 595-2250

PROVISIONAL PATENT APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. §1.53(c).

Docket Number	01656.0010.PZUS00

INVENTOR(S)/APPLICANT(S):

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
1. Garlich 2. Durden	Joseph Donald	R. L.	Westfield, IN Indianapolis, IN

TITLE OF THE INVENTION

PTEN INHIBITORS FOR SENSITIZATION OF CANCER CELLS

CORRESPONDENCE ADDRESS

Attention: IP Prosecution
HOWREY SIMON ARNOLD & WHITE, LLP
Box 7, 2941 Fairview Park Drive
Falls Church, VA 22042

STATE	VA	ZIP CODE	22042	COUNTRY	USA

ENCLOSED APPLICATION PARTS (check all that apply)

_X_ Specification Number of Pages: 6

_X_ Drawing(s) Number of Sheets : 29

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL PATENT APPLICATION (check one)

A check or money order is enclosed to cover the Provisional Patent Application filing fees	Pro-visional Filing Fee Amount(s)	$ = $80.00
X **The Commissioner is hereby authorized to charge any deficiencies in filing fees, or credit overpayments, to Deposit Account Number: 08-3038**		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE _____ Date July 20, 2004

TYPED or PRINTED NAME Teddy C. Scott, Jr., Ph.D. REGISTRATION NO. 53,573 (if appropriate)

PROVISIONAL PATENT APPLICATION FILING ONLY

PTEN INHIBITORS FOR SENSITIZATION OF CANCER CELLS

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention is generally related to the modulation of apoptosis. More specifically, the present invention is related to methods of sensitizing cells to apoptosis.

2. Description of Related Art

[0002] The PI3K/PTEN pathway is a critical non-redundant pathway controlling angiogenesis, apoptosis, and proliferation. Activation of the PI3K pathway, either constitutively or via growth factor stimulation leads to phosphorylation of AKT, and activation of multiple other downstream signals critical to cell survival. Inhibiting such an important target could potentially confer significant anti-tumor effects.

DETAILED DESCRIPTION

[0003] The present invention is related to the use of PTEN inhibitors to enhance the sensitivity of cancer cells to inhibitors of the PI3 kinase. PTEN inhibitors are administered for a period of time sufficient to make the cancer cells more dependant on PI3 kinase mediated signals including, but not limited to, downstream signals such as p-AKT and mTOR. Once administration of the PTEN inhibitor is discontinued, the cancer cells experience a disruption or alteration in the PI3 kinase pathway. The disruption if the PI3 kinase pathway may be anywhere along the pathway including upstream growth factor receptors. The cancer cells are not able to adjust quickly enough and succumb to resulting pro-death signal conditions or at least disruptions in the pro-survival signal conditions.

[0004] The methods of the present invention are also able to stimulate cancer "stem cells" to enter into a state whereby they are susceptible to treatment using a PI3 kinase pathway inhibitor. Cancer stem cells are believed to be the reason that cancer is resistant to treatment because they are quiescent and thus resistant to chemo and radiation therapy.

[0005] The present invention is also related to the use of PTEN inhibitors in conjunction with medical procedures that are known to result in elevated risk of adverse side effects derived from cellular apoptosis. Representative examples of such procedures include, but are not limited to, open heart surgery, surgery in general, invasive cardiovascular procedures, and general

anesthesia. PTEN inhibitors are administered for a period of time sufficient to prevent apoptosis to a desired extent. The PTEN inhibitor may be administered before, during, after or a combination thereof with respect to the procedure.

Example 1

[0006] Small molecule PTEN inhibitors are administered to patients suffering from cancer via a route of administration including, but not limited to, oral, i.v., sub-cutaneous, i.v. drip, intramuscular, nasally as aerosol, dermal patch, mucous exposure, etc as compatible conventional formulations or as drug delivery modalities such as slow release formulations, depots, liposomes, microparticles, nanoparticles, and degradable and/or targeted versions thereof. The inhibitors are administered for a limited period of time sufficient to convert at least 10% of cancer cells from basal levels of phospho-Akt to at least 10% increased levels of phospho-Akt.

[0007] The patients are then withheld from further treatment with PTEN inhibitors and subsequently treated with inhibitors of the PI3 Kinase pathway including, but not limited to, singly or in combination: a) growth factor regulators and growth factor receptor inhibitors (such as antibodies and/or receptor trysine kinase inhibitors-Irressa); b) PI3 kinase inhibitors (including for examples specific isoforms, e.g. p110alpha isoform) such as but not limited to LY294002 (and prodrugs thereof as described in U.S. Patent Application No. 10/818,145, which is incorporated by reference), wortmanin, and other known inhibitors (such as disclosed by Piramed); c) PDK inhibitors; d) Akt inhibitors; e) mTOR inhibitors (such as but not limited to rapamycin, CCI-779, etc); f) mdm2 inhibitors; g) nfkb inhibitors; h) integrin antagonists; i) proteosome inhibitors; j) tyrosine kinase inhibitors; k) HIF inhibitors; l) and the like.

Example 2

[0008] Patients suffering from cancer are treated as described in Example 1, except the administration of the PTEN inhibitor and the PI3 Kinase pathway inhibitor overlap to a small extent to minimize toxicity to normal cells.

Example 3

[0009] Patients suffering from cancer are treated as described in Examples 1 and 2, except without using the PI3 kinase pathway inhibitor but instead using any single or combination of chemotherapy or radiation therapy or immunotherapy or other oncology methodology that

because of the prior exposure to the PTEN inhibitor becomes capable of then adversely affecting the survival or viability or reproduction ability of the cancer cells and cancer stem cells.

1-9-04: Discussions with Dr. Dan Durden at his home in atlanta - I called him at home.
During the call he and I talked about the concept he has been mulling over as consultant for Cancer Chem (Nonsenature). The concept is to use a PTEN inhibitor (such as our discovered PTEN inhibitors CC1523 CC1589, CC1617 etc) to treat a patient with cancer so that the cancer cells see inhibition of PTEN for a controlled period of time (not so long that it stimulates them to become significantly more difficult to treat) which makes them dependant or more dependant on the PI3kinase-AKT pathway (this may be performed also in the presence of chemotherapy or radiation therapy - optionally), after which (after removal of PTEN inhibitor) the patients cancer cells are exposed in vivo to a PI3kinase pathway inhibitor (such as CC1101 (=LY294002) or other PI3kinase inhibitors; Akt inhibitors, mTOR inhibitors such as Rapamycin etc.) again optionally with chemo or radiation treatment simultaneously or optimally timed to maximize cell death.

As refinements to this concept we envision selective delivery (i.e. targeted delivery by ERB, integrin targeted nanoparticle, liposomal, bone targeted, etc.) via targeting mechanisms to selectively trick cancer cells into being sensitive to being killed.

BEST AVAILABLE COPY

Continued on Page X

Read and Understood By

Joseph R Garlich 1-9-04 Barry Wreckam 1/19/04
Signed Date Signed Date

For signal transduction therapeutics to work must find the cell pathway you are targeting to ensure a requirement for a specific signal relay pathway
i.e PI-3 kinase
ERK
SRC family kinase
Syk kinase.

Then use another pharmacologic antagonist to modify & abruptly "stop" this signal relay process.

So inhibit PTEN using 1523 or 1584... for 24-48° then treat with CC1101 a CC1126 or nanoparticle 1107.

Or treat with CSK (C-SRC-kinase) inhibitor then scan with SFK inhibitor

The concept is to create a greater requirement for signaling pathway in the tumor or stromal (tumor-related) cell then take away this signal.

Our data demonstrate that this maneuver of "set up" tumor or endothelial cell for destruction, using small molecule inhibitors of specific signaling pathways is the key to disrupting "wiring".

Continued on Page

Read and Understood By

Signed 11/28/2003 Date

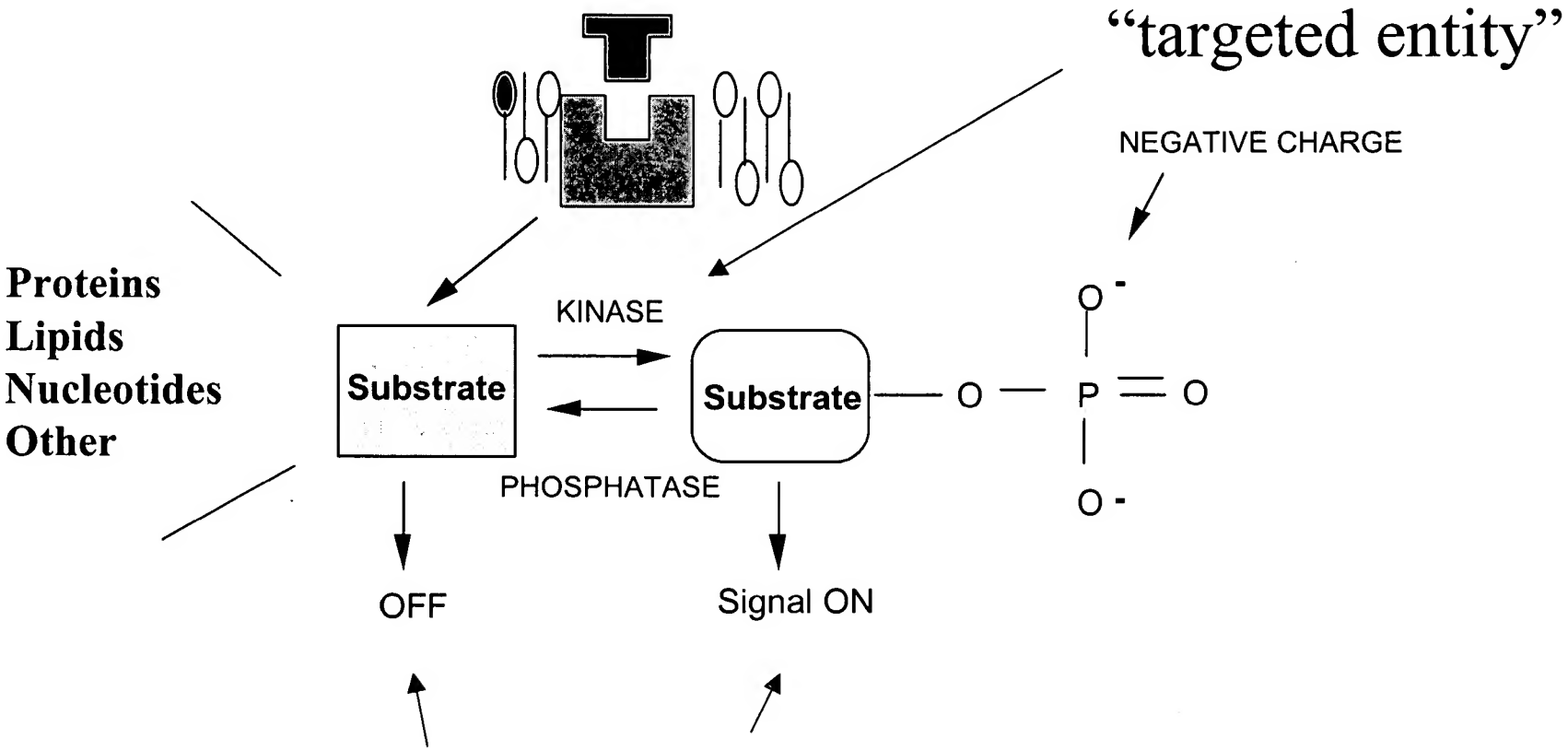
Signed 1/22/04 Date

CLAIMS

1. A method of sensitizing cancer cells to an inhibitor of the PI3 kinase pathway comprising administering to a patient in need of such treatment a PTEN inhibitor.

2. A method of treating apoptosis associated with a medical procedure comprising administering to a patient in need of such treatment a PTEN inhibitor.

"PHOSPHORYLATION"

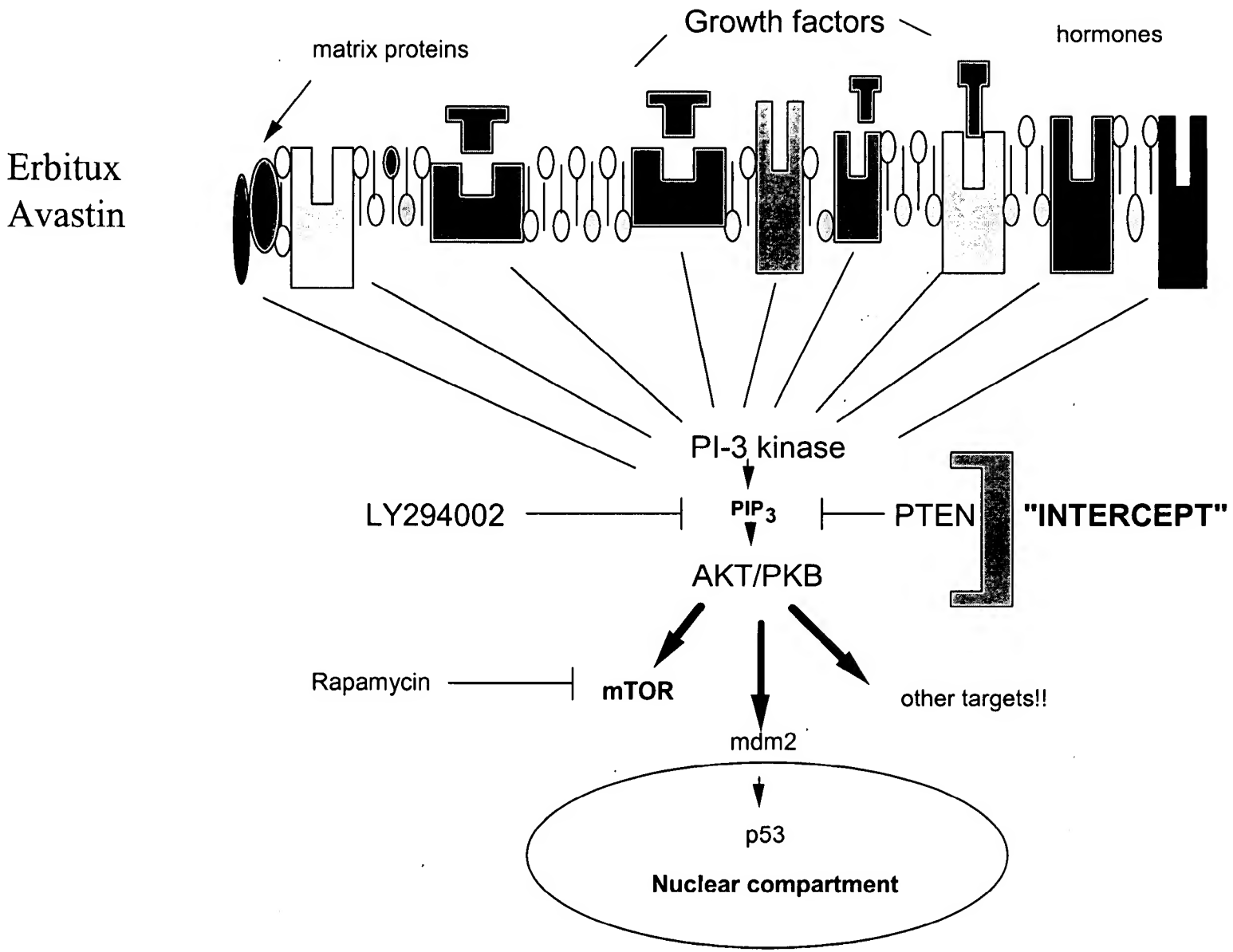


"Yin and Yang" of Cell Signaling

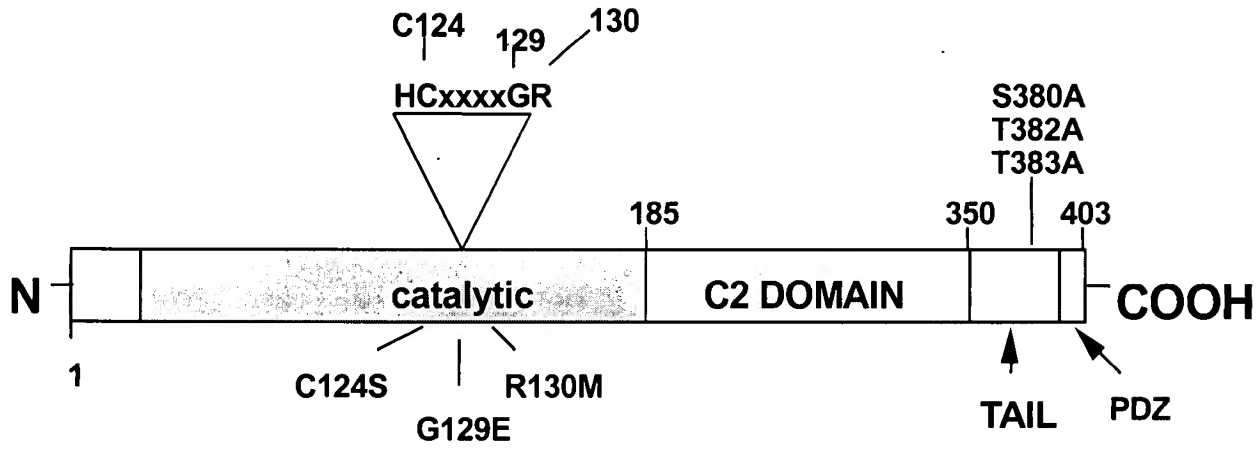
Intercept Point in Mammalian Signaling

- Point in mammalian signaling where the pathways via multiple cell surface receptor pathways converge.
- Nonredundant "cant get around it"
- If targeted "knockout" lethal phenotype.
- More likely to exert marked control in case of catastrophic phenotype (cancer and massive apoptosis, grade VI, GVHD)
- Not so useful for manipulation of subtle pathologic phenotypes (SCD, arthritis, etc.)

Intercept Concept- Target nonredundant component

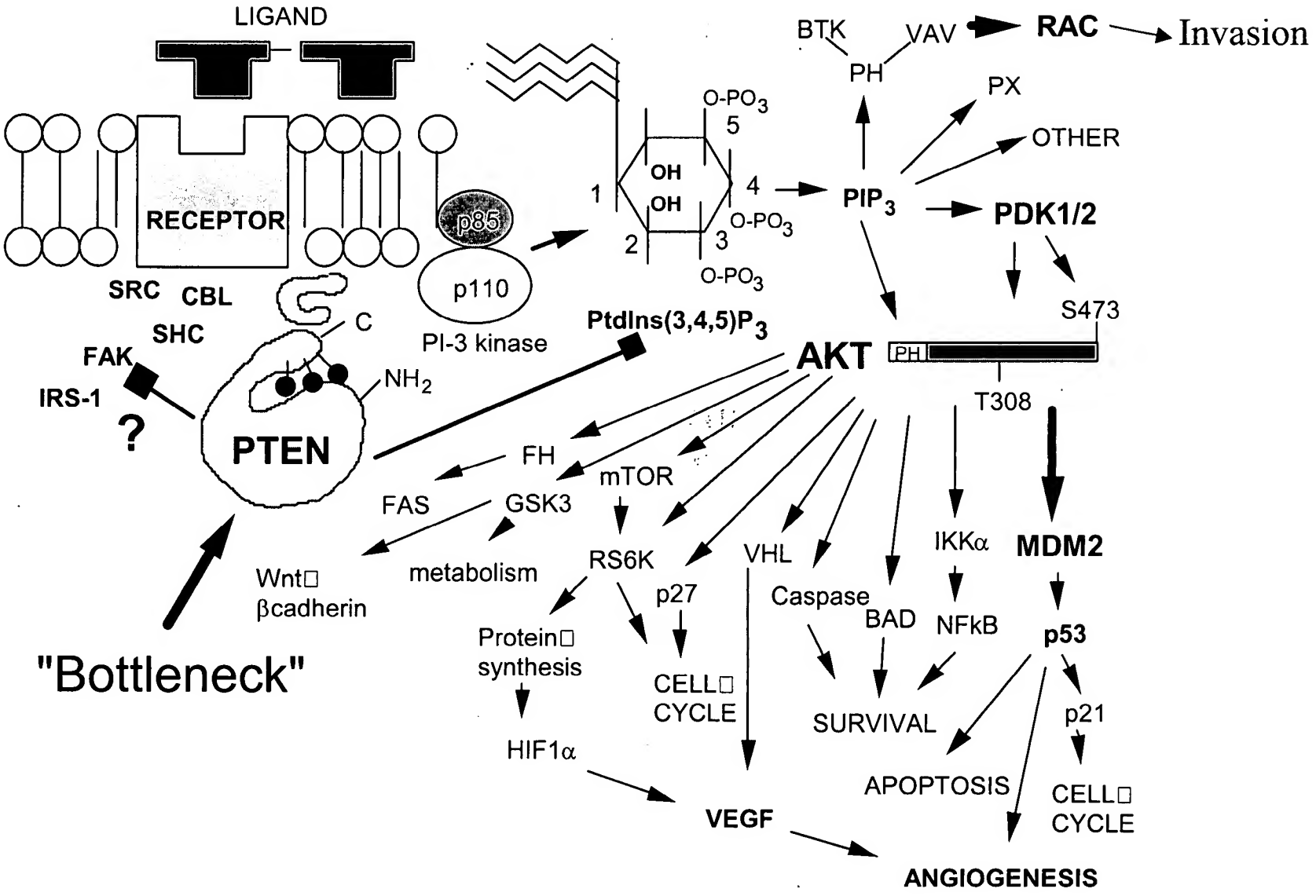


PTEN

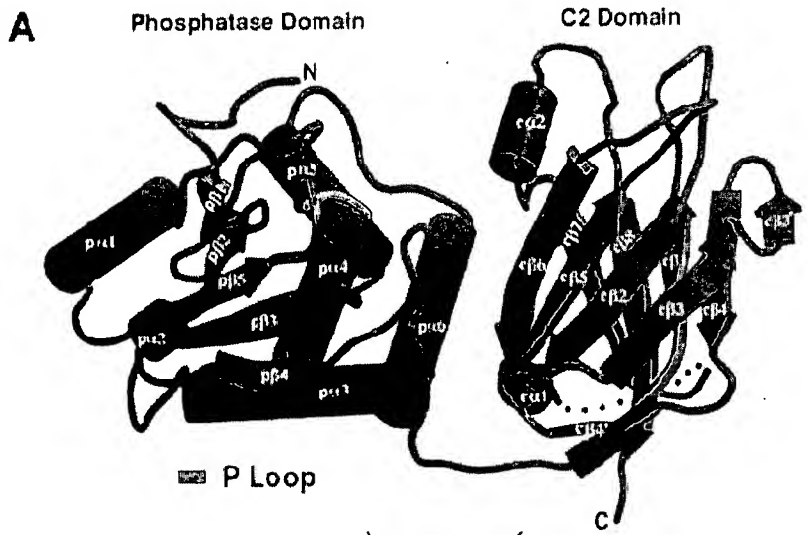


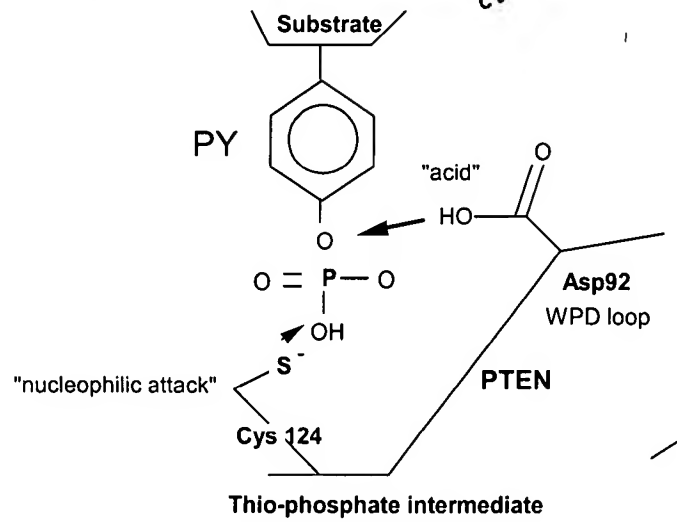
1) Dual specificity protein and lipid phosphatase
2) PTEN -/- lethal (ED 10.5), PTEN -/+ tumors multiple organ systems.
2) Only phosphatase which dephosphorylates D3 position inositol ring (PIP_3 regulation)
3) Tumor suppressor gene (Glioblastoma, Prostate, etc.
4) Familial cancer syndromes (Cowdens s.)
5) Malignant "angiogenic" tumors associated PTEN mutations. (30% pediatric, 40% adult GBMs)
6) Potential role PI-3 kinase in angiogenesis?

PTEN/AKT Signaling Axis



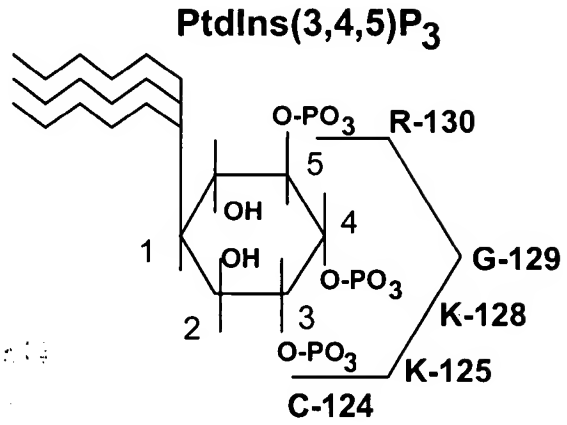
Crystal Structure PTEN

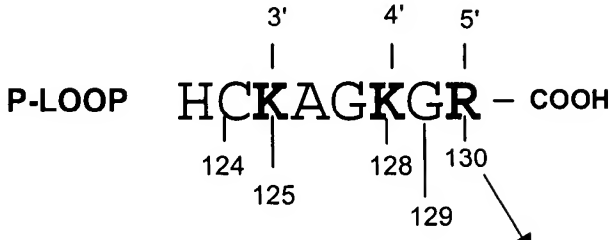




"Drug target"

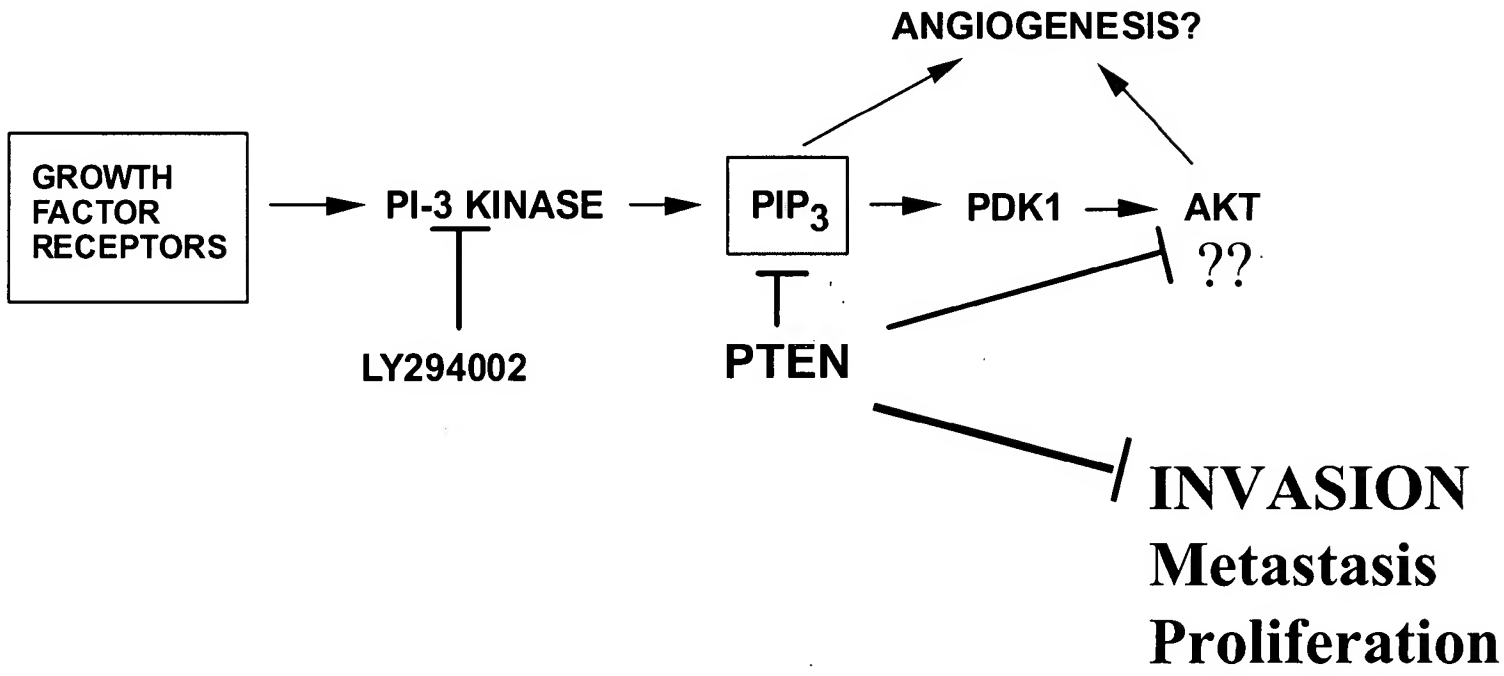
PTEN Active Site

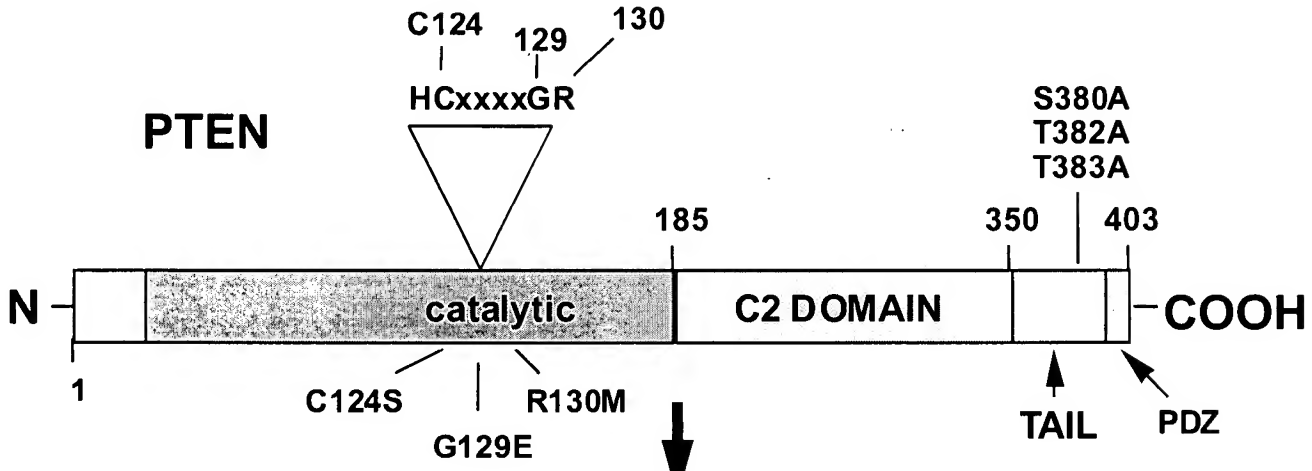




R130M
"dead"

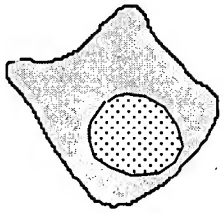
G129E
lipid dead


ANGIOGENESIS?
GROWTH FACTOR RECEPTORS
PI-3 KINASE
PIP3
PDK1
AKT
??
LY294002
PTEN
INVASION
Metastasis
Proliferation



pBABE-Puro vector
- PTEN G129E — **PIP_3 dead**
- PTEN WT
- PTEN R130M — **catalytic dead**

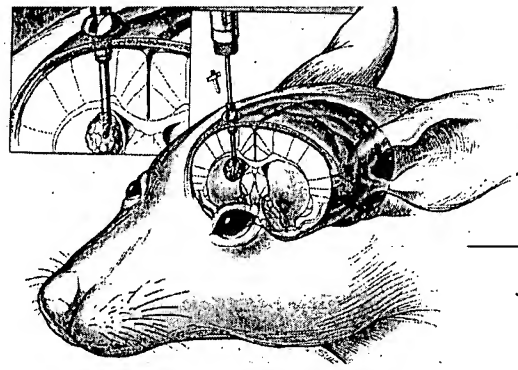
(PTEN null)(p53 mut) U373MG, U251MG, LNZ308 +



U87MG GBM (PTEN null)(p53 WT)

Stable clones

Stereotactic Orthotopic Injection tumor cell into R Frontal lobe



Survival

Invasion

Angiogenesis

Microarray analysis

Biochemistry

(AKT, RAC)

α PTEN blot

PTEN

β-actin

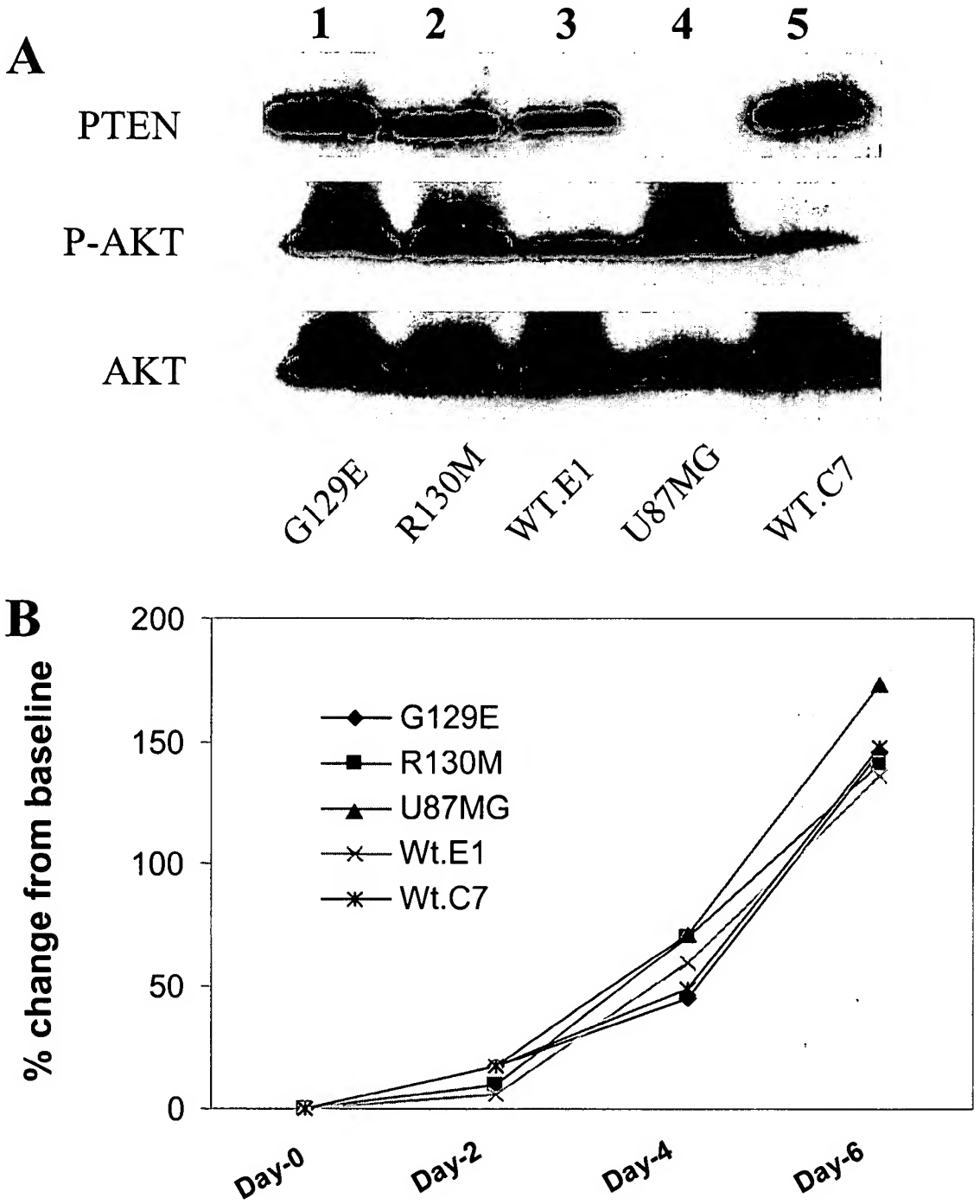
WT.E1

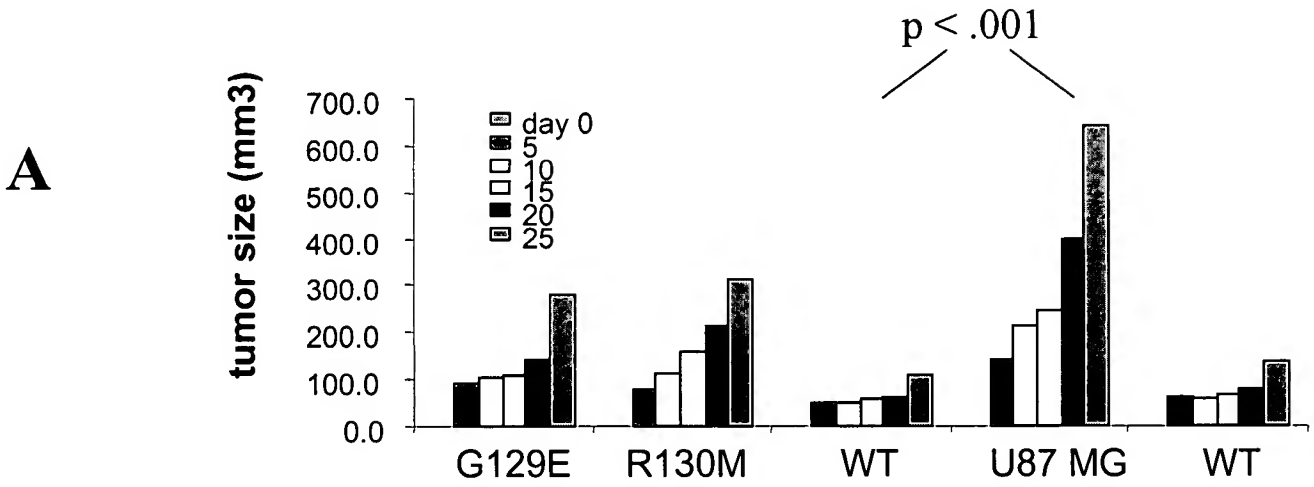
U87MG

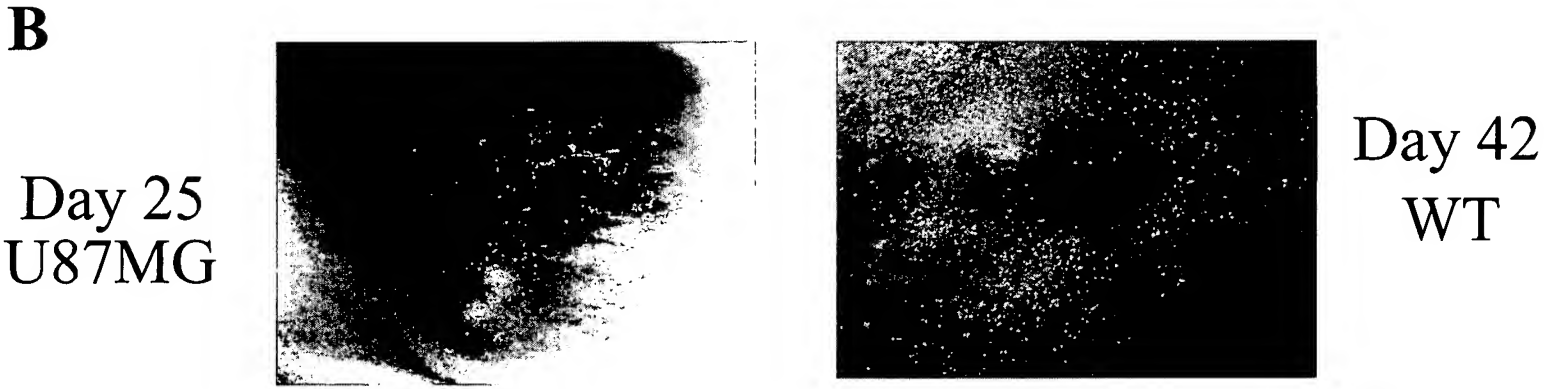
RM..C7

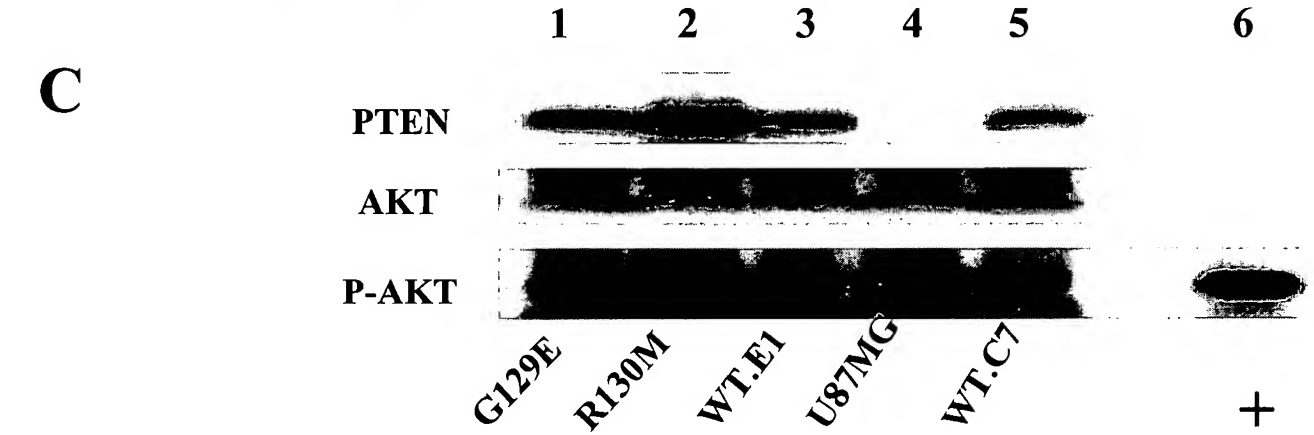
Astrocytes

Normal Brain

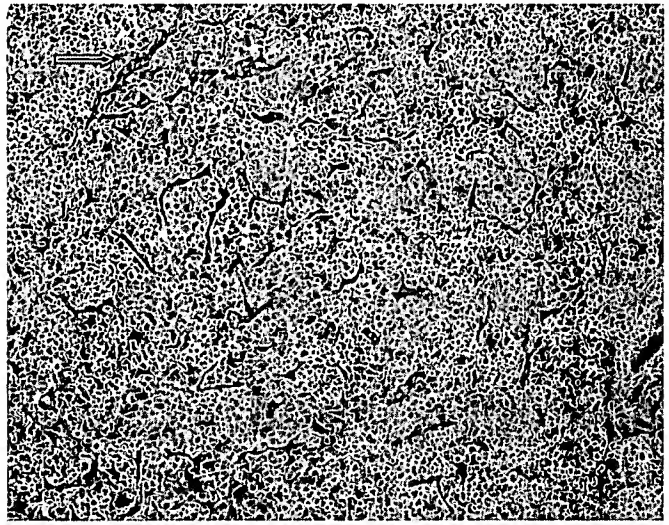

A
1
2
3
4
5
PTEN
P-AKT
AKT
G129E
R130M
WT.E1
U87MG
WT.C7
B
% change from baseline
200
150
100
50
0
G129E
R130M
U87MG
Wt.E1
Wt.C7
Day-0
Day-2
Day-4
Day-6


A
p < .001
tumor size (mm3)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
day 0
5
10
15
20
25
G129E
R130M
WT
U87 MG
WT

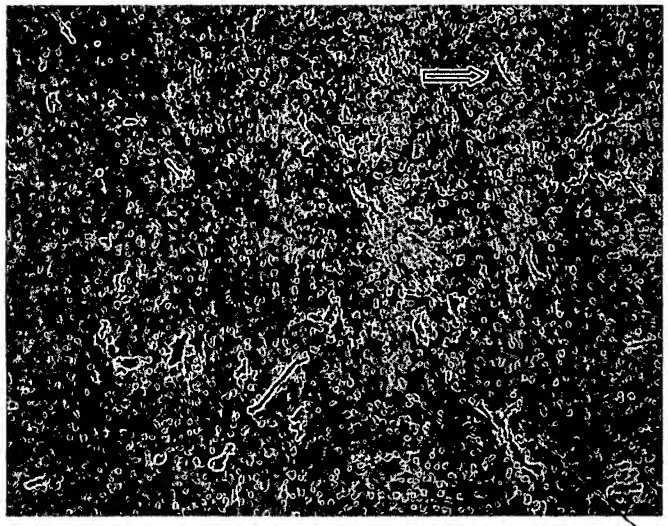

B
Day 25
U87MG
Day 42
WT


C
1
2
3
4
5
6
PTEN
AKT
P-AKT
G129E
R130M
WT.E1
U87MG
WT.C7
+

A



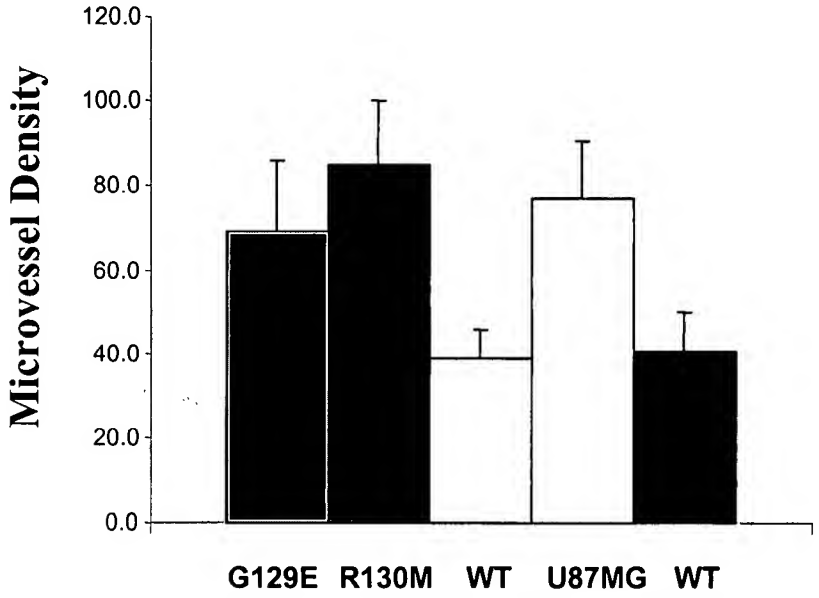
B



αCD31 IHC

"microvessel"

C

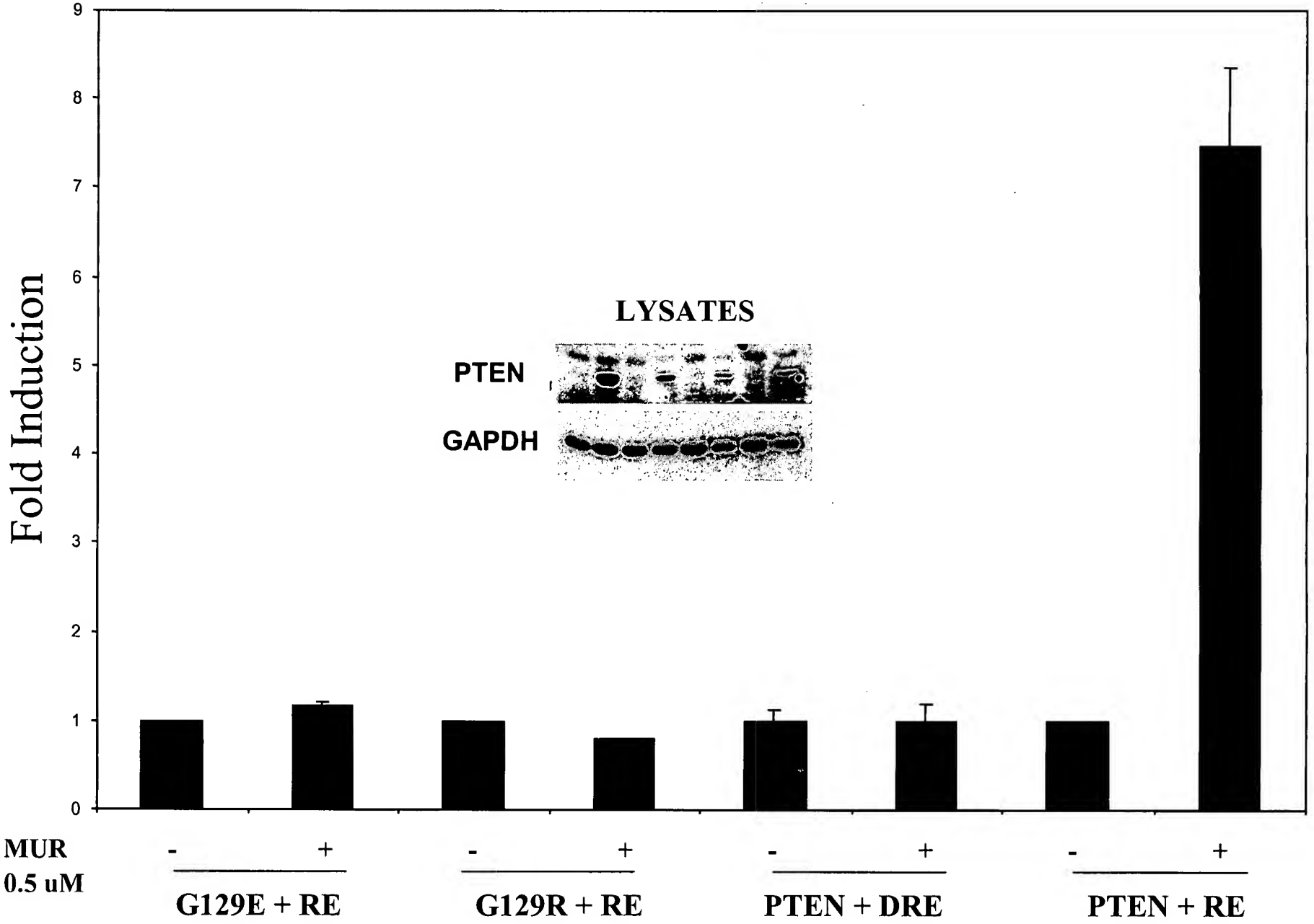


"lipid dead"

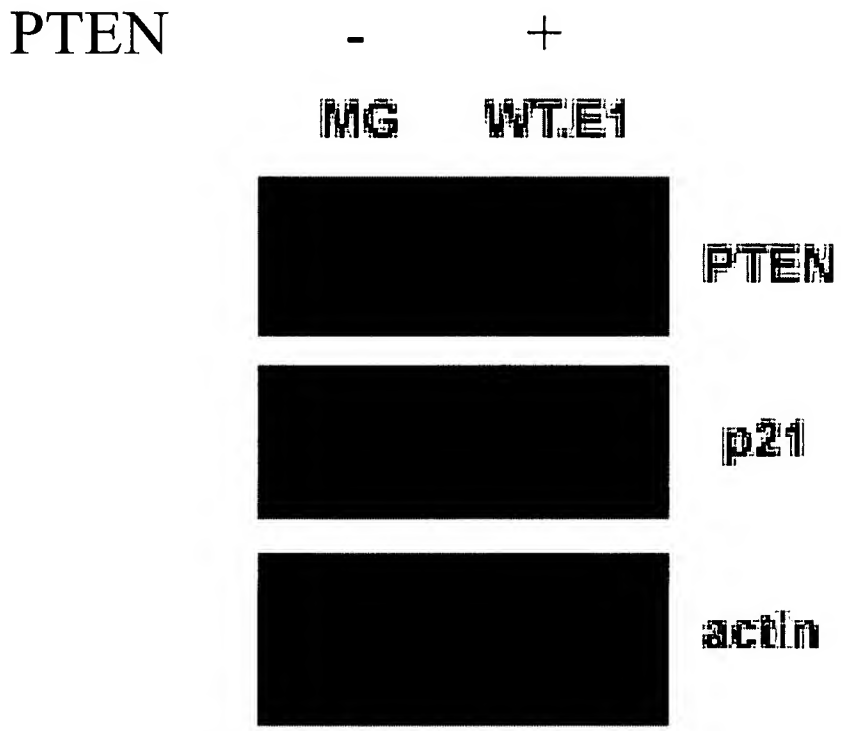
$p < .001$

PNAS, 2001

PTEN regulates p53 activity!!



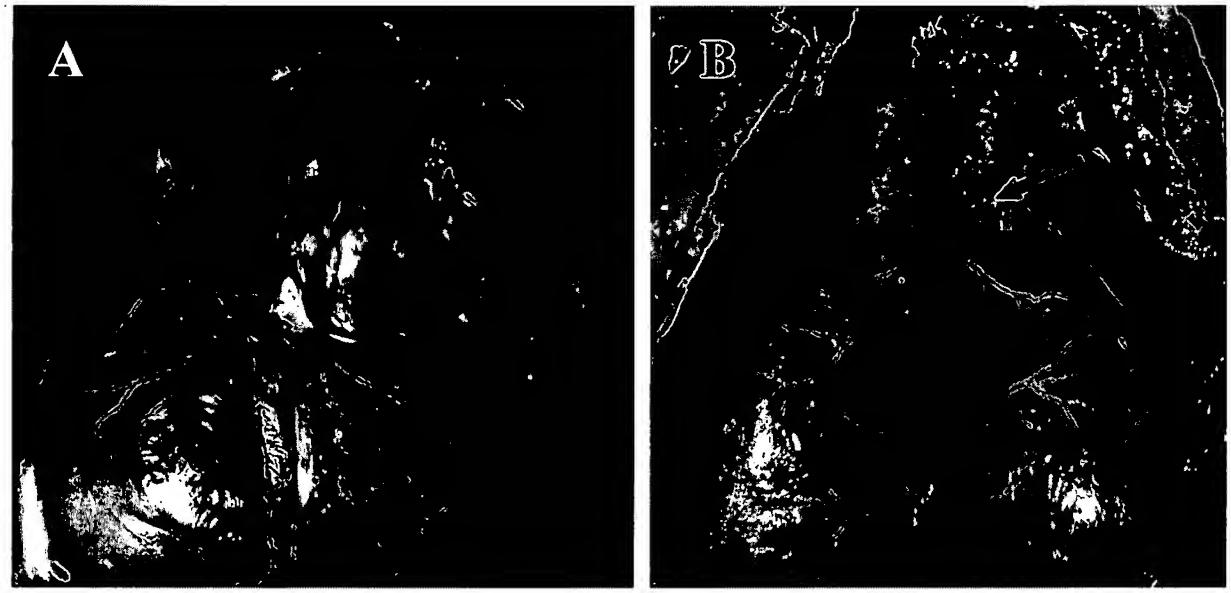
PTEN regulates $p21^{waf-1}$ expression



Day 25

→

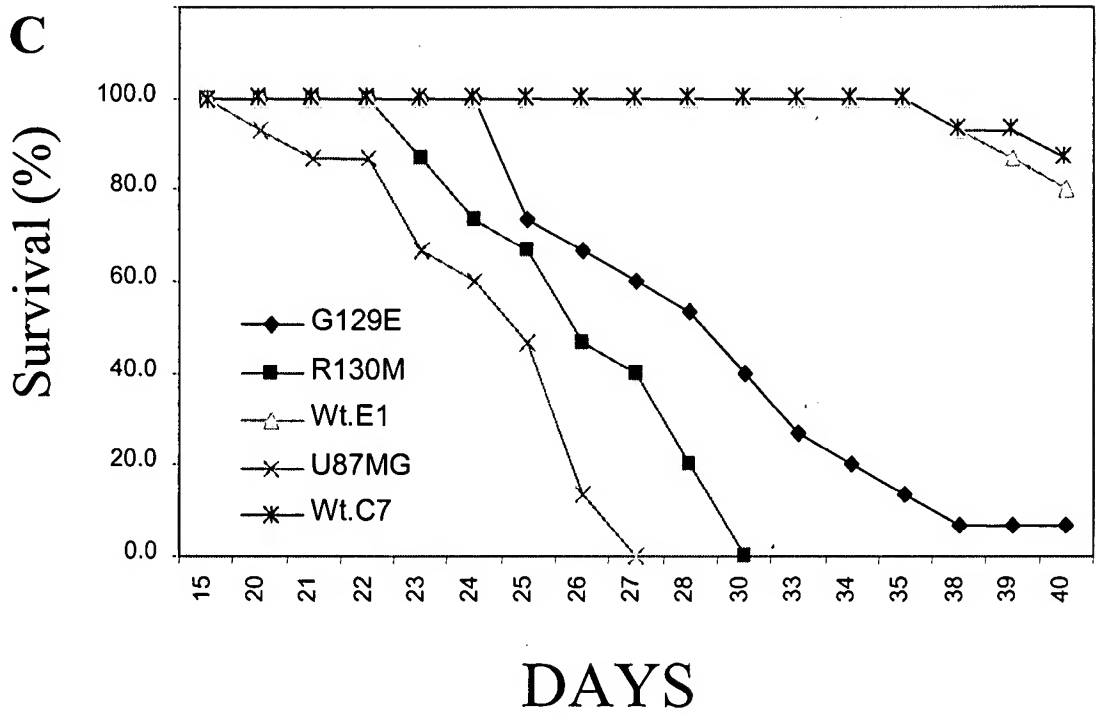
"null"



PTEN
WT

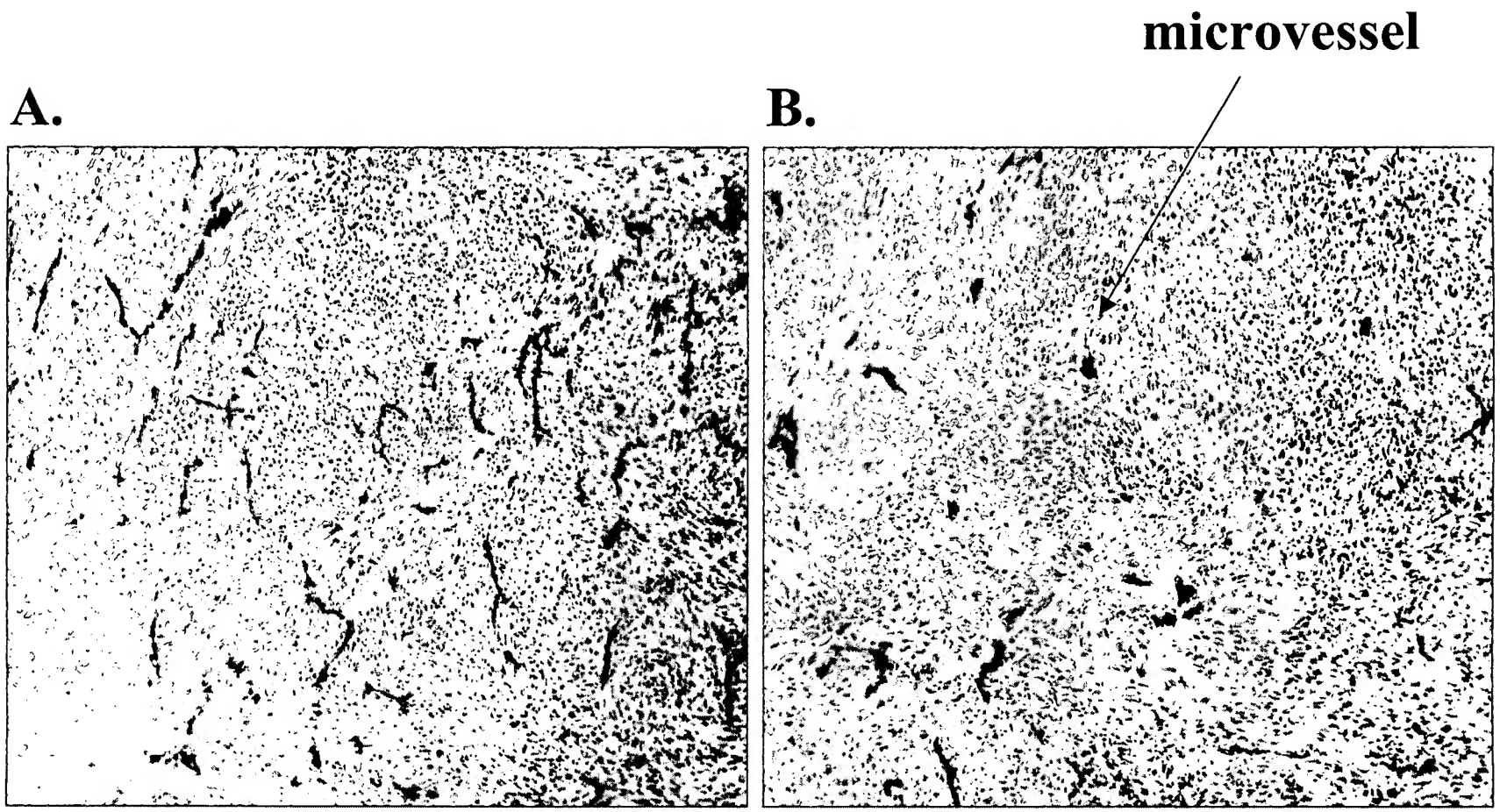
← Day 42

C

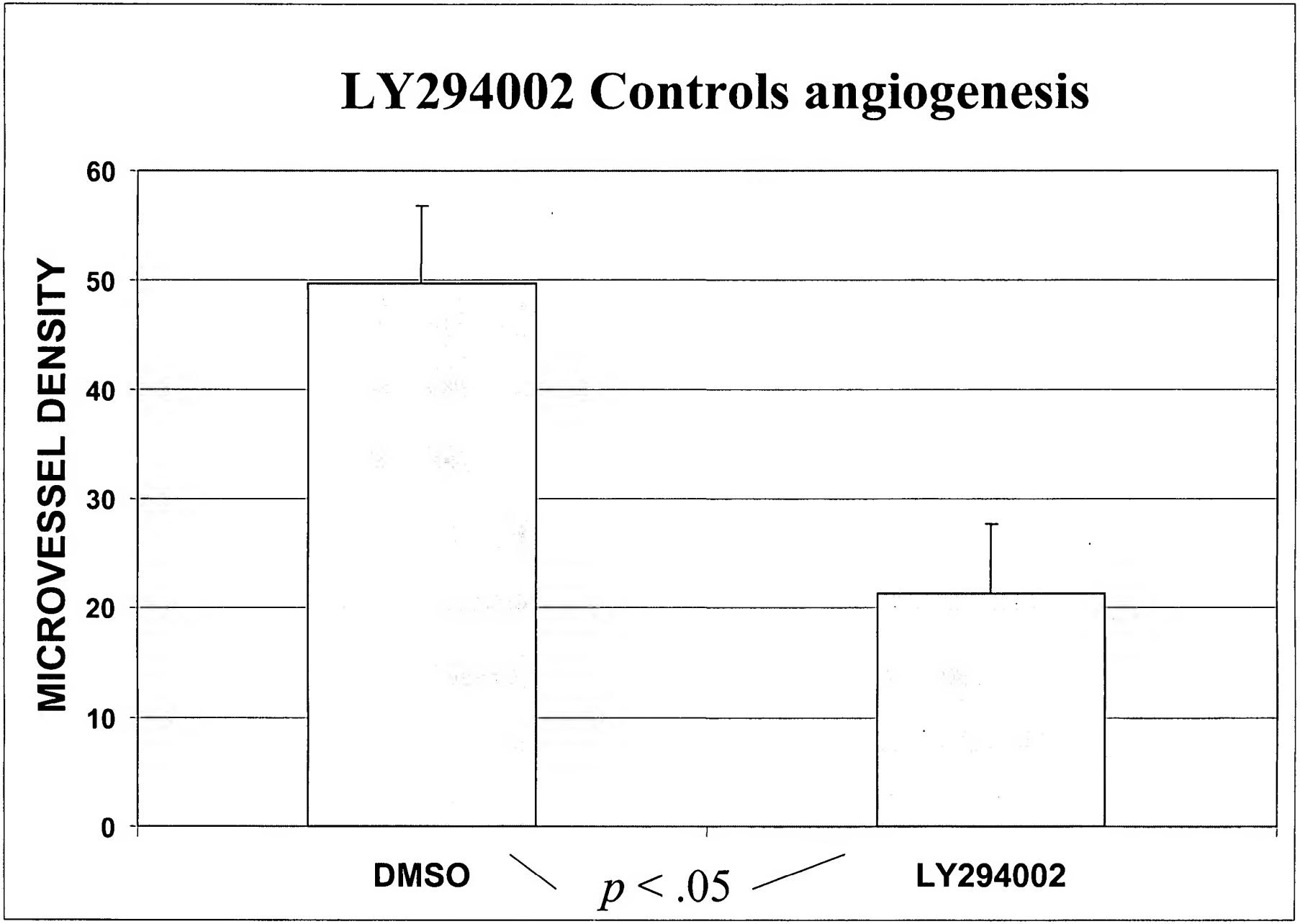


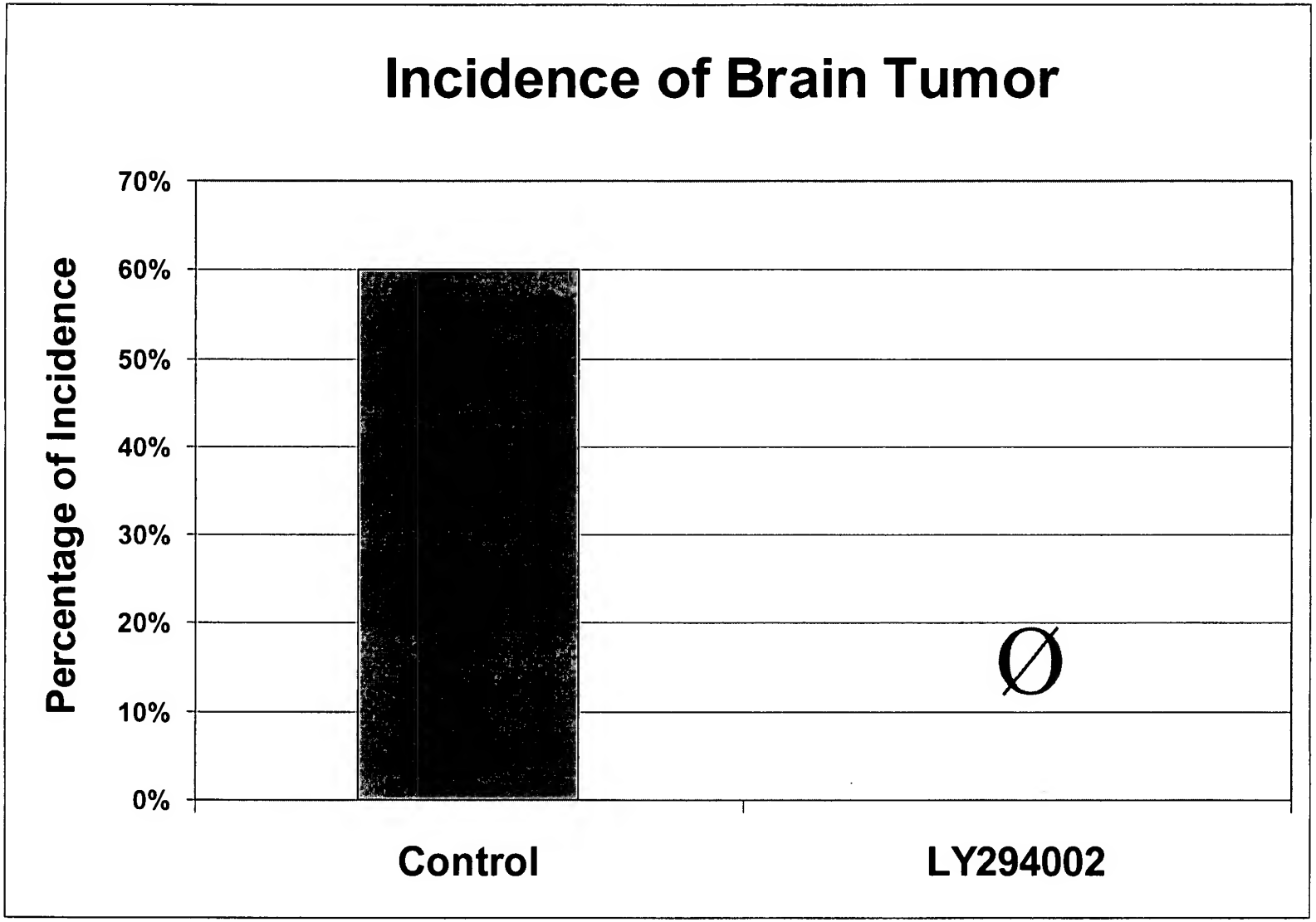
$p < .0001$

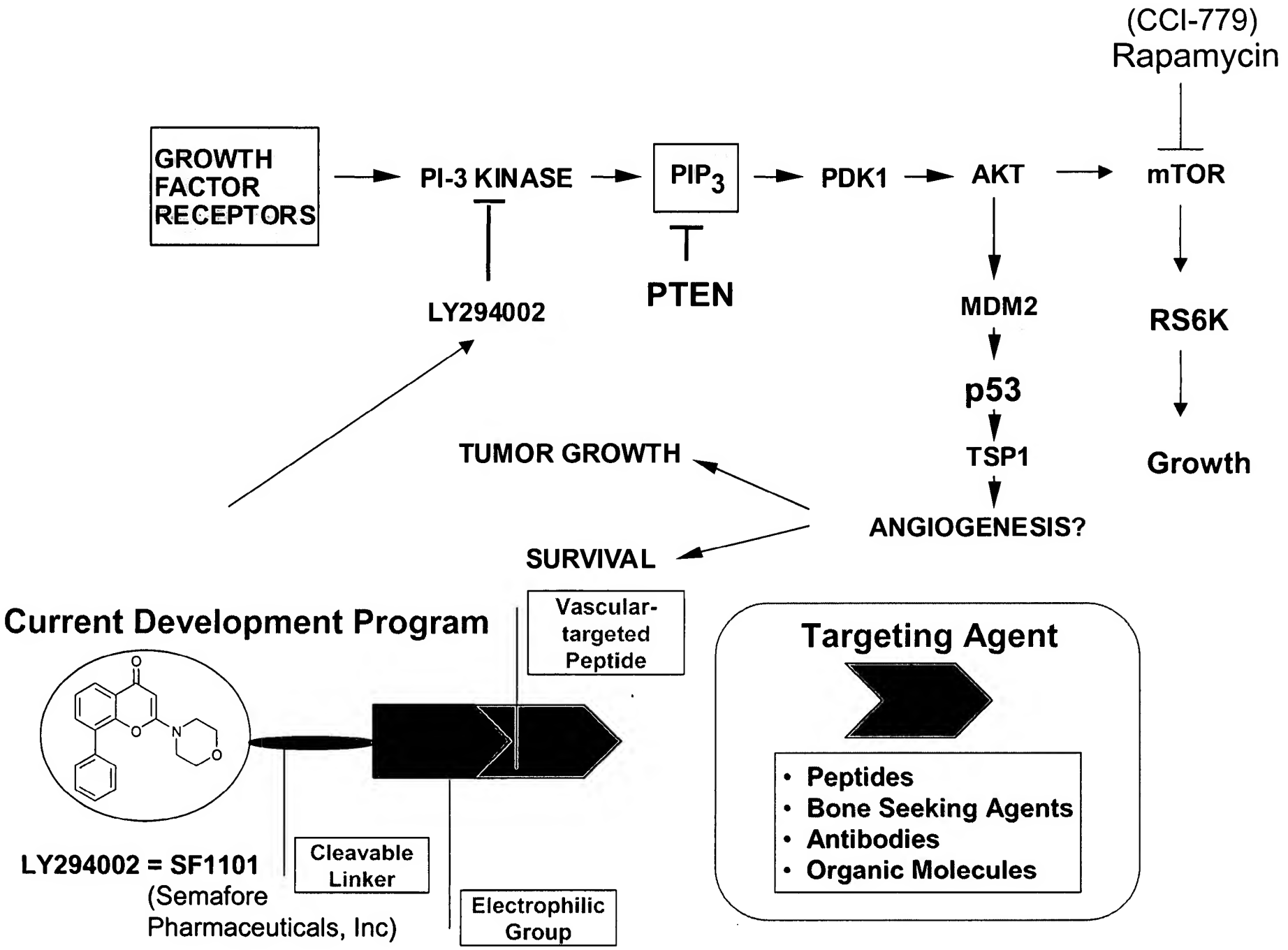
PNAS, 2001



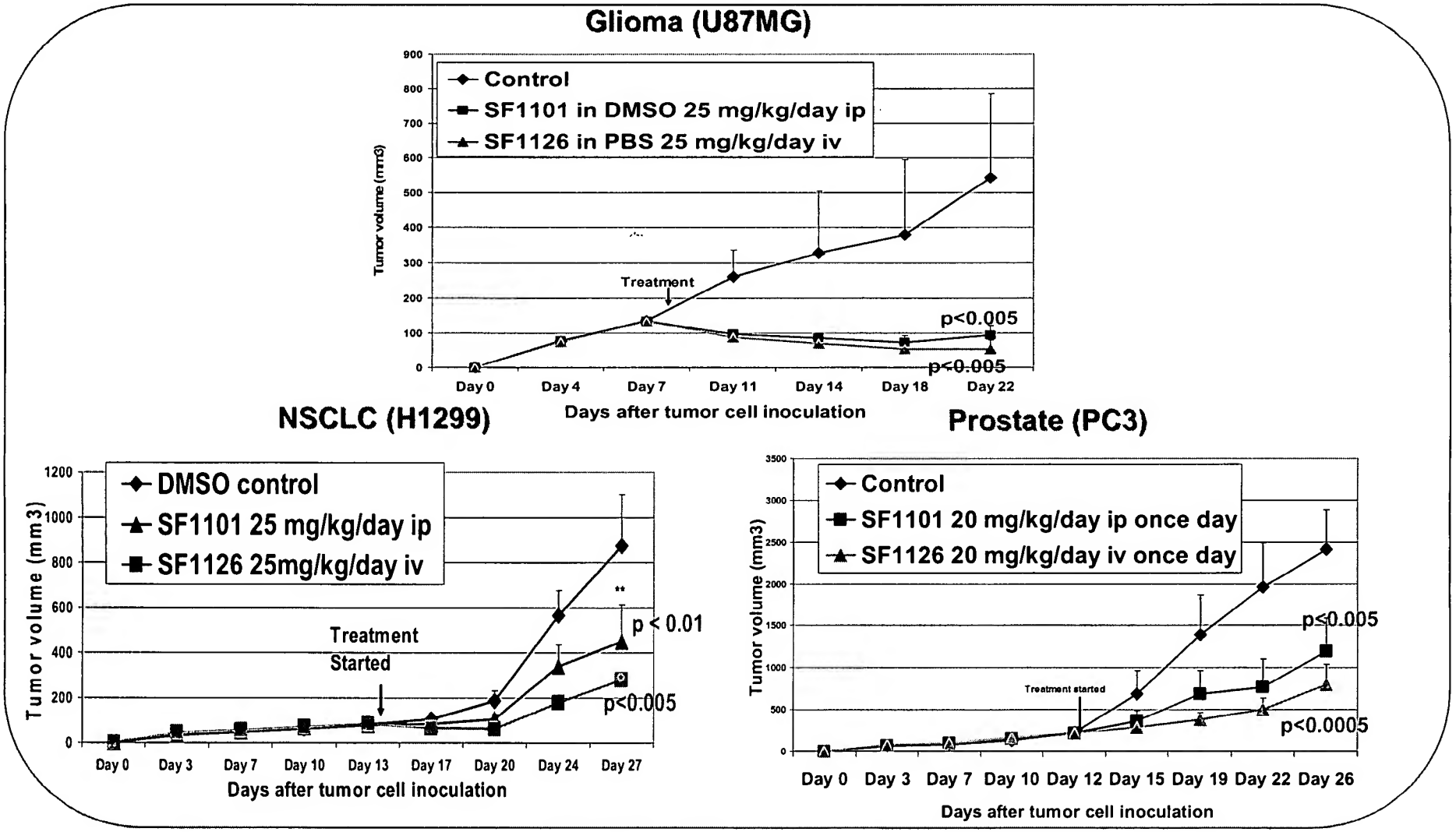
αCD31 IHC


LY294002 Controls angiogenesis
MICROVESSEL DENSITY
60
50
40
30
20
10
0
DMSO
$p < .05$
LY294002


Incidence of Brain Tumor
Percentage of Incidence
70%
60%
50%
40%
30%
20%
10%
0%
Ø
Control
LY294002


(CCI-779)
Rapamycin
GROWTH FACTOR RECEPTORS
PI-3 KINASE
PIP3
PDK1
AKT
mTOR
LY294002
PTEN
MDM2
RS6K
p53
TSP1
Growth
TUMOR GROWTH
ANGIOGENESIS?
SURVIVAL
Current Development Program
Vascular-targeted Peptide
Targeting Agent
• Peptides
• Bone Seeking Agents
• Antibodies
• Organic Molecules
LY294002 = SF1101
(Semafore Pharmaceuticals, Inc)
Cleavable Linker
Electrophilic Group

Antitumor activity of SF1126

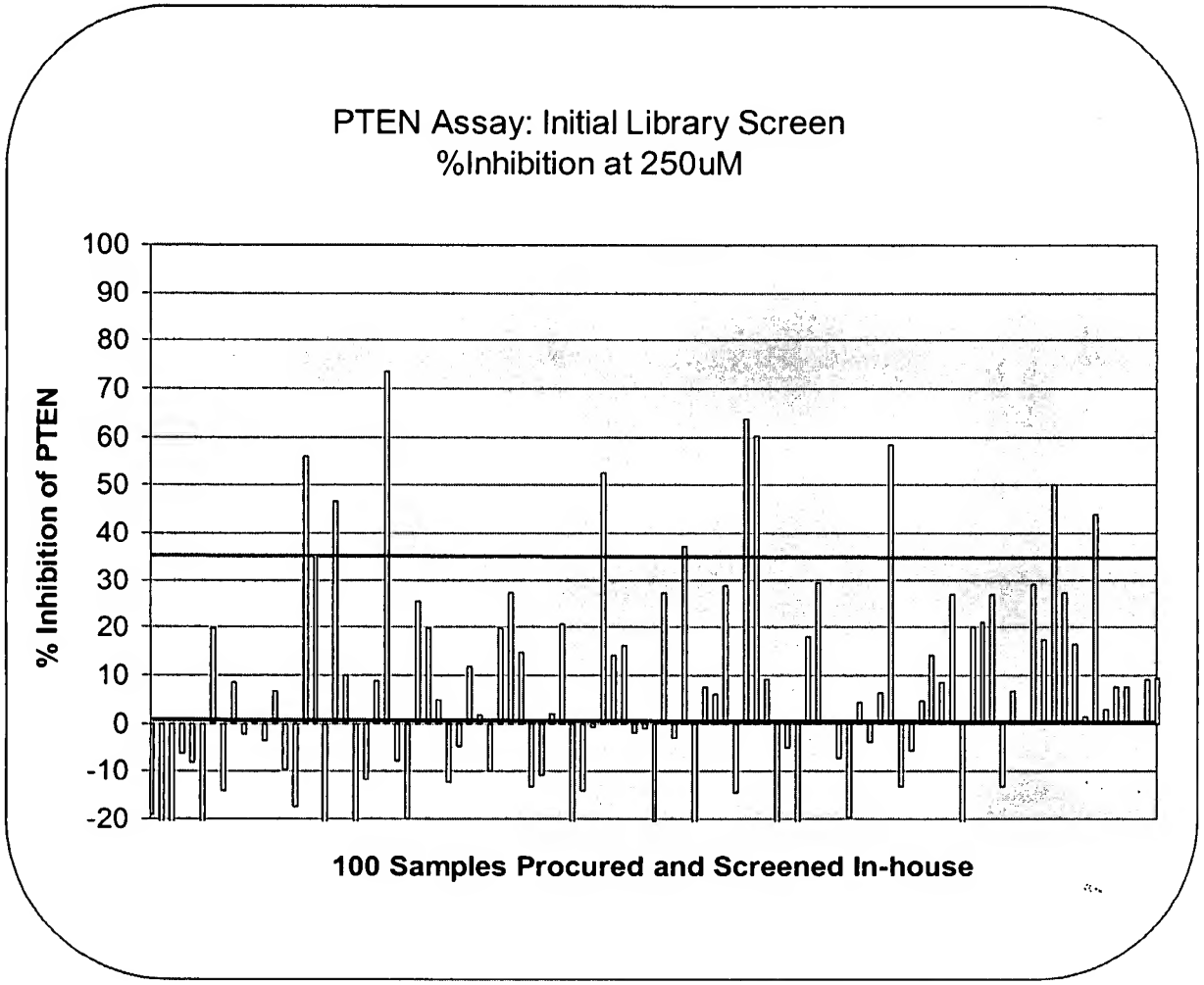


(courtesy of Semafore Pharmaceuticals, Inc)

PTEN inhibitors why?

- Tumor cells most sensitive to PI-3 kinase inhibitors PTEN deficient
- Cell survival and proliferation dependent on PI-3 kinase-AKT axis
- PTEN loss tumor cells angiogenesis and invasion dependent on PI-3 kinase-PIP3
- PTENi is pharmacologic method to train tumor cells to require PI-3 kinase
- Subsequent inhibition of PI-3 kinase,AKT, mTOR will result in big problem for tumor cells.

PTEN Inhibitors??

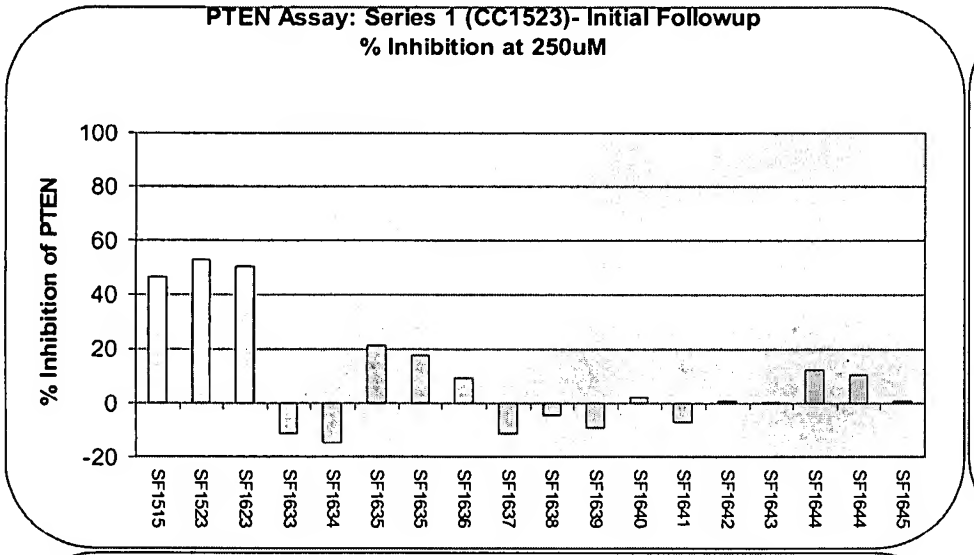


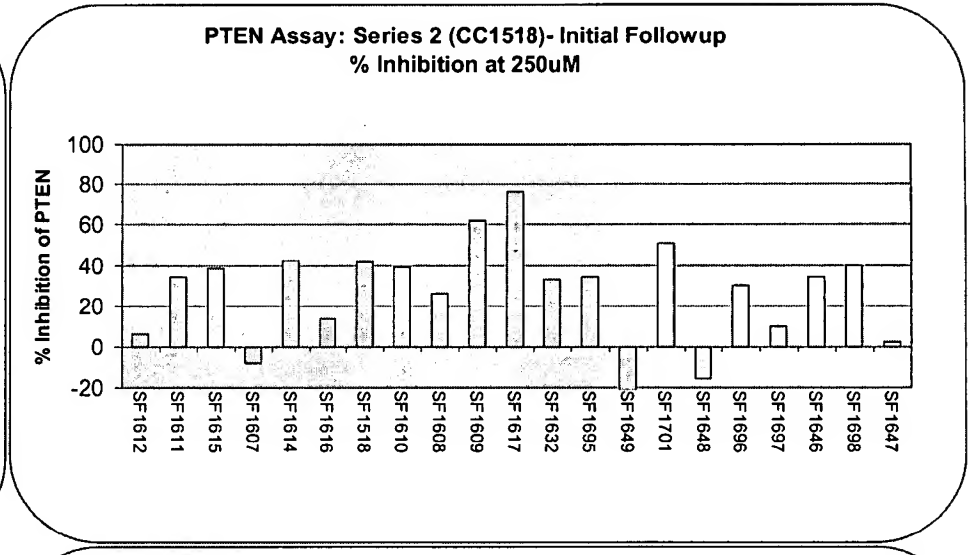
(courtesy of Semafore Pharmaceuticals, Inc)

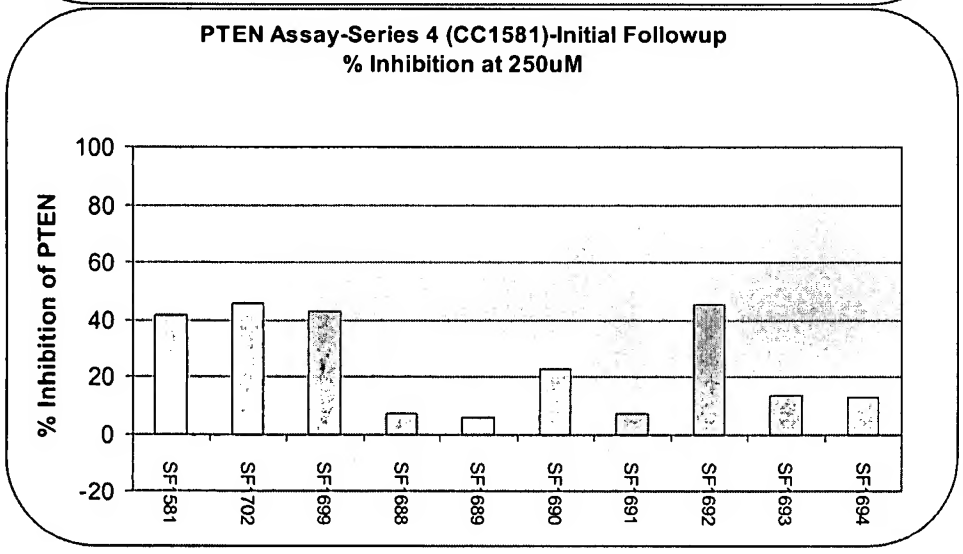
1. **In Silico – ChemNavigator**
   a. Exclusive
   b. 9 million screened
   c. Top 3000 selected
   d. Library of 100
2. **In vitro – Semafore Bioassay Group**
   a. 250 uM in Level 1
   b. PIP3 as substrate
3. **35% inhibition were confirmed and rerun**
4. **Enhanced Activity Noted**
5. **IC50s determined**
6. **Selectivity analyzed**
7. **Proof of concept initiated (in vitro/in vivo)**

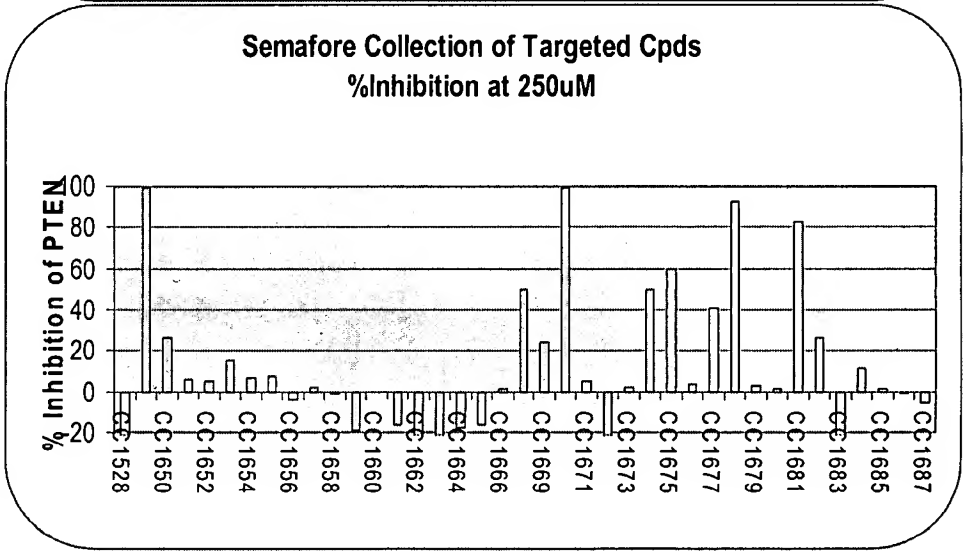
PTEN inhibitors

- PTEN hits divided into four (4) series currently

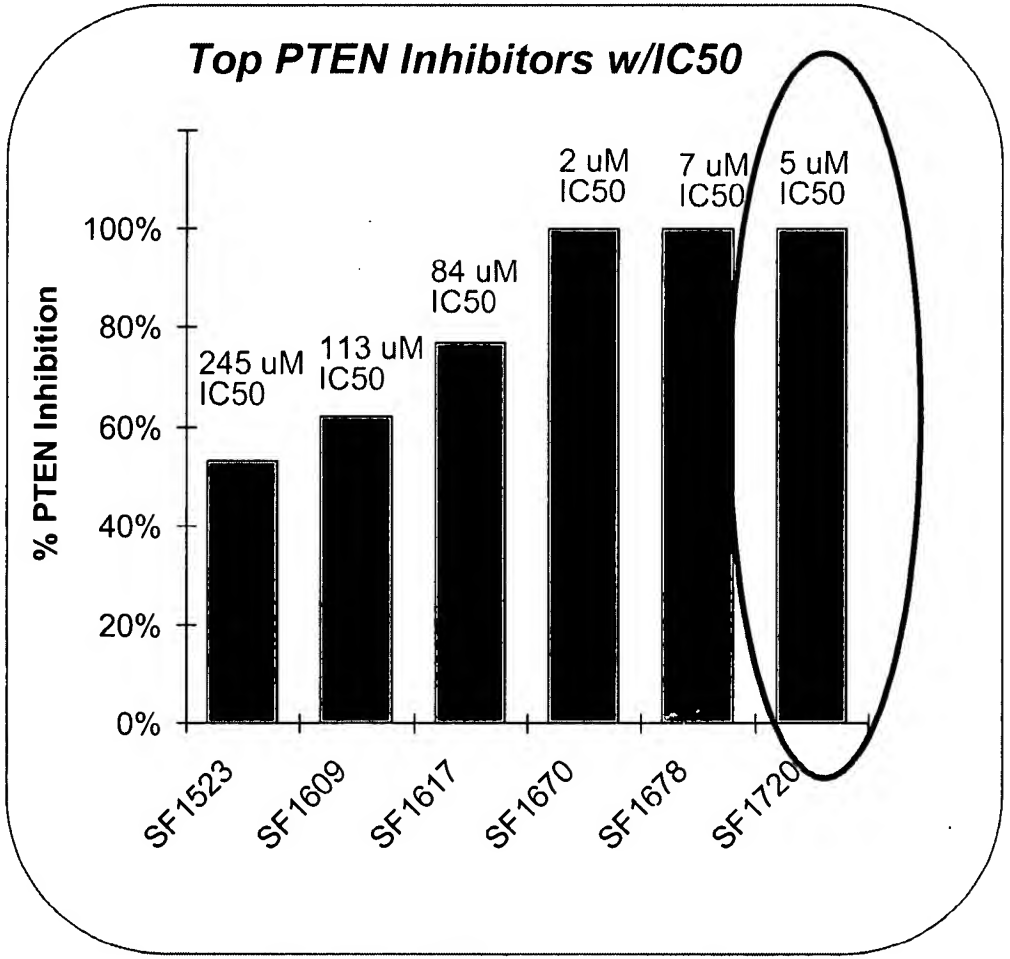




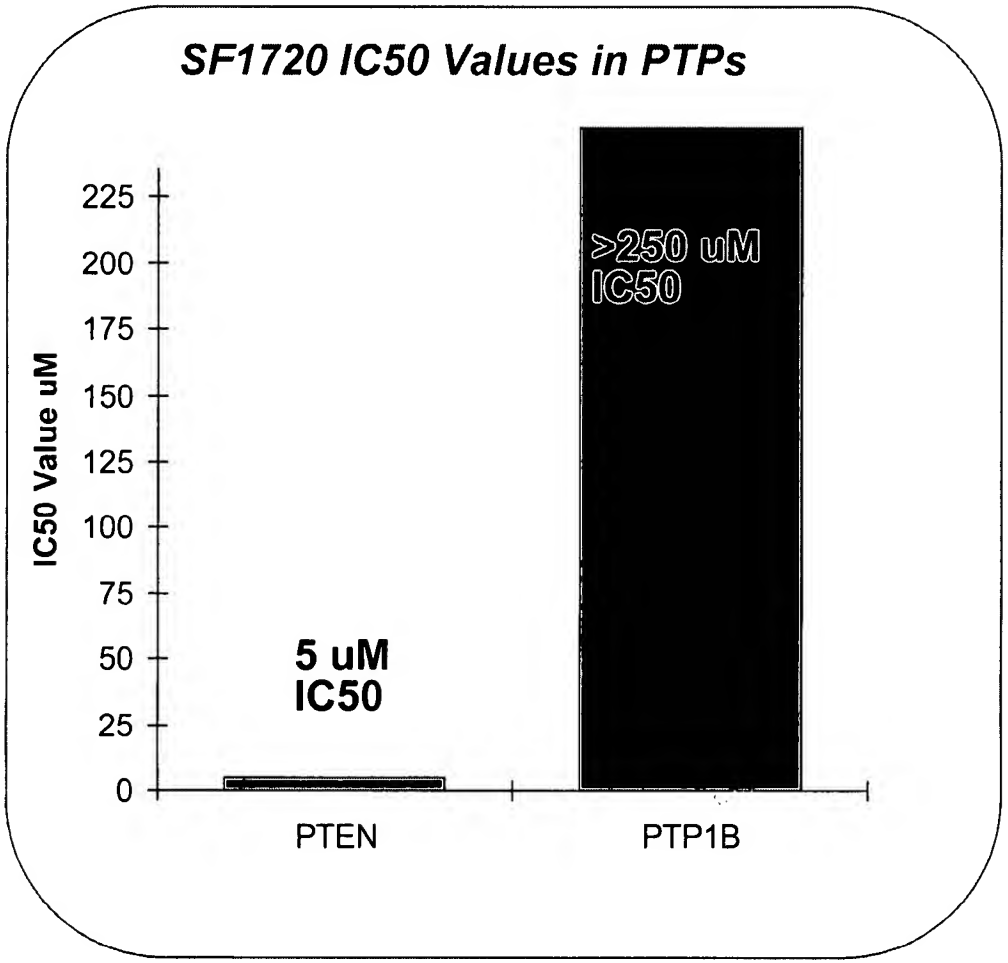




Proof Of Concept — First Known PTEN Inhibitor (Potency)

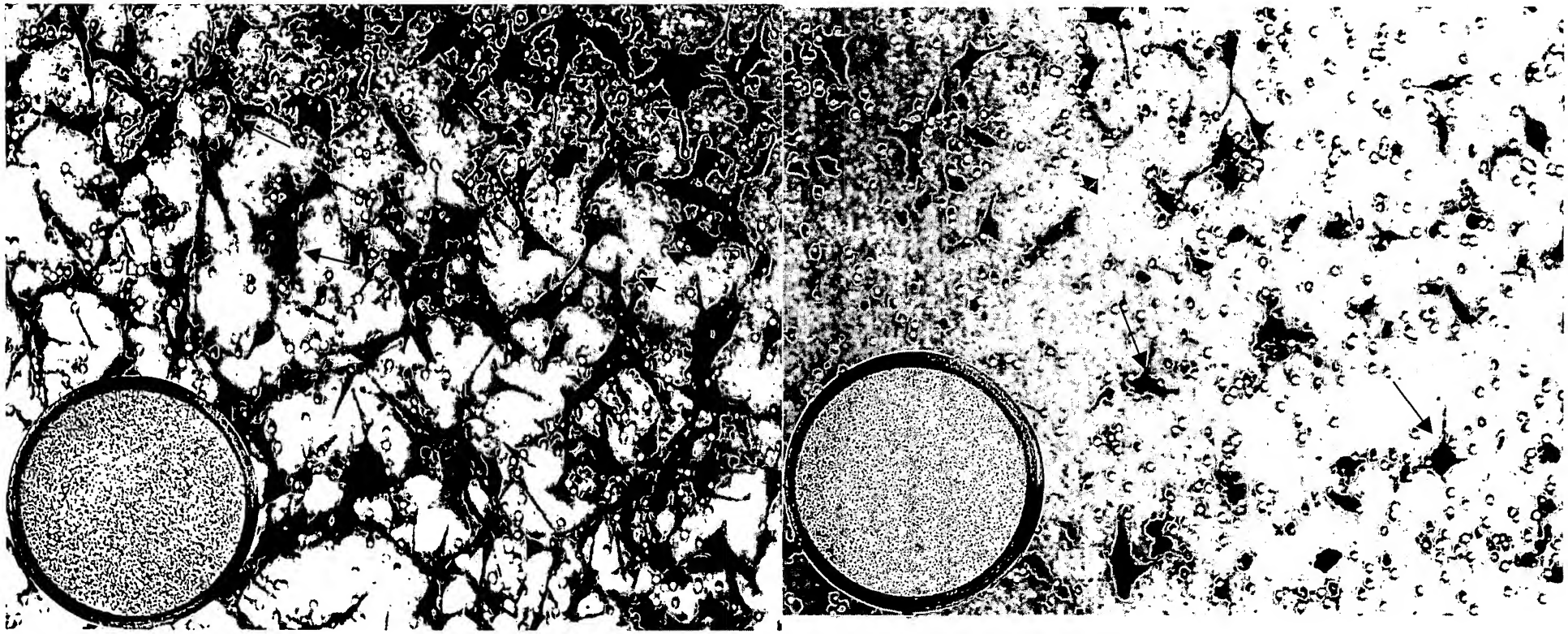


Selectivity – SF1720



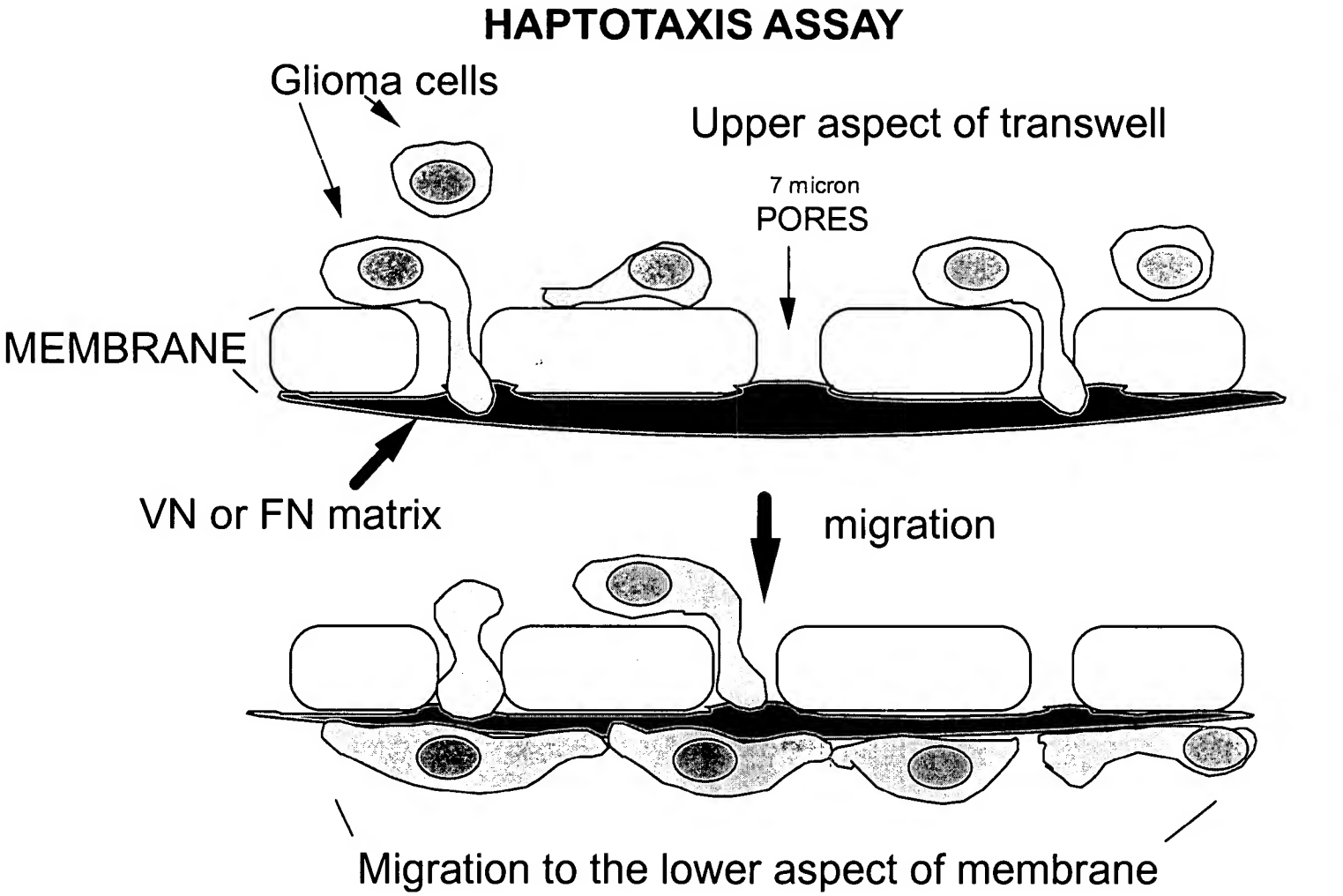
D.

U87MG Migration on VN (αvβ3)

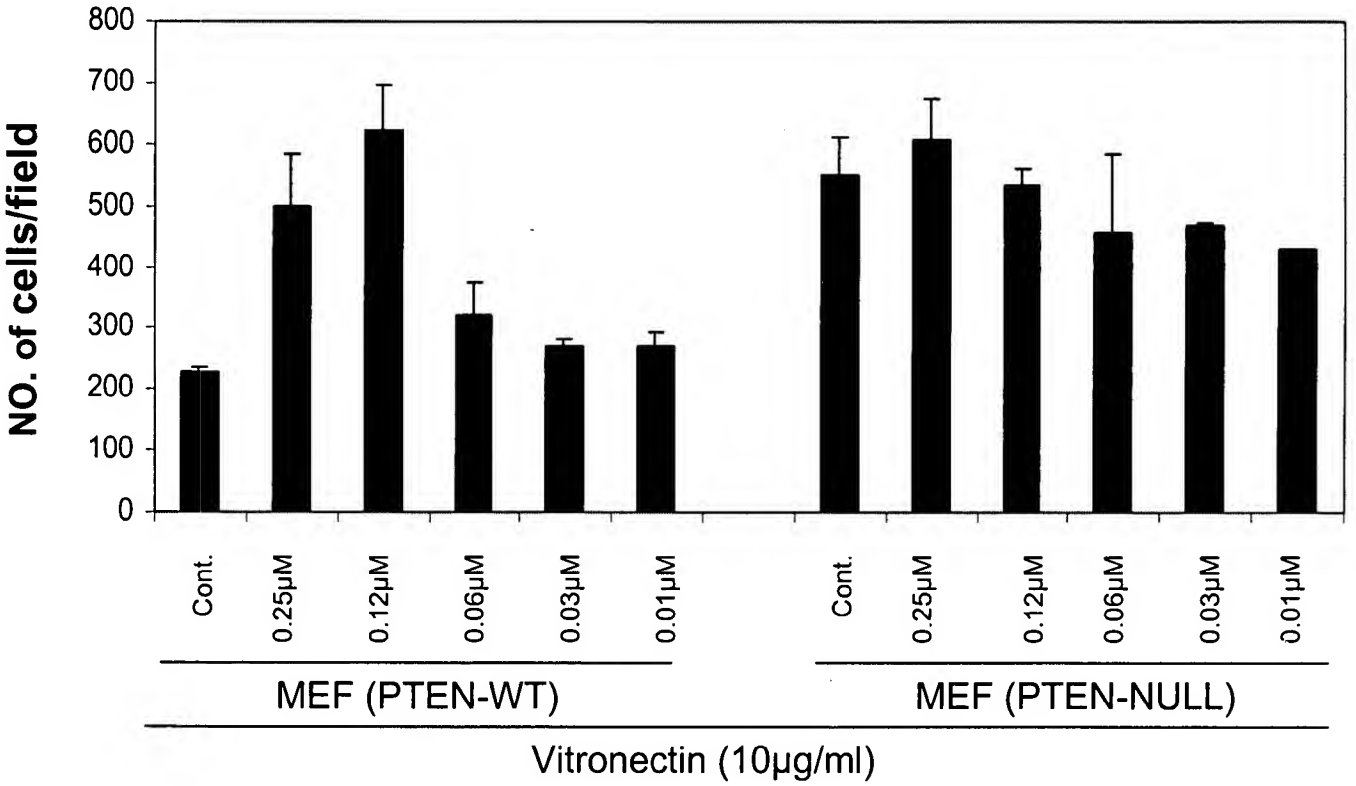


PTEN / NULL

PTEN / WT

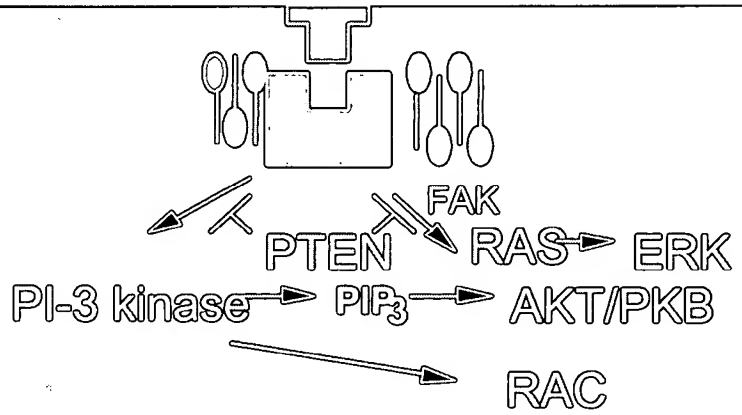

HAPTOTAXIS ASSAY
Glioma cells
Upper aspect of transwell
7 micron
PORES
MEMBRANE
VN or FN matrix
migration
Migration to the lower aspect of membrane

Role of PTEN inhibitor on integrin directed migration



Dt.04-08-04

Conclusions



- PTEN reconstitution blocks glioma growth and angiogenesis *in vivo*.
- PTEN and PAN-PI-3 kinase inhibitors antiglioma and antiangiogenic activity *in vivo*.
- Vascular targeted PAN-PI-3 kinase inhibitor (SF1126) has efficacy without toxicity in glioma xenograft model.
- We describe the first specific small molecule inhibitor for PTEN phosphatase activity.
- Apply our PAN-PI-3 kinase inhibitors to the manipulation of tumor proliferation, angiogenesis and chemoradiosensitivity *in vivo*.